



#### FIGURE 1A

#### NUCLEOTIDE SEQUENCE OF NS2/3 (810-1206) ST (SEQ ID. No. 1)

atggaccggg agatggctgc atcgtgcgga ggcgcggttt tcataggtct tgcactcttg accttgtcac catactataa agtgctcctc gctaggctca tatggtggtt acagtattta atcaccagag tegaggegea ettgeaagtg tggateece eteteaatgt teggggagge cgcgatgcca tcatcctcct cacgtgcgca gtccacccag agctaatctt tgacatcacc ttcgtgcgtg cgcaggggct cattcgtgcg tgtatgttgg tgcggaaggc tgcggggggt cattatgtcc aaatggcctt catgaagcta gctgcgctga caggtacgta cgtttatgac catctcactc cattgcagga ttgggcccac gcgggcctac gagaccttgc agtggcggta qaqcccqtca tcttctctga catggaggtc aagatcatca cctggggggc ggacaccgcg gcatgcgggg acatcatttc aggtctgccc gtctccgctc gaaggggaag ggagatactc ctgggaccgg ccgataattt tgaagggcag gggtggcgac tccttgcgcc catcacggcc tactcccaac agacacgggg cctacttggt tgcatcatca ccagcctcac aggccgggac aagaaccagg tcgaggggga ggttcaagtg gtctccaccg ctacacaatc tttcctggcg acctgcgtca acggcgtgtg ttggactgtc ttccatggcg ccggctcaaa gaccttggcc ggccccaaag gcccaatcac ccagatgtac actaatgtgg accaggacct cgtcggctgg caggegeece etggggegeg etecatgaca ceatgeacet geggeagete ggacetetat ttqqtcacqa qacatgccga cgtcattccg gtgcgccggc ggggcgacag tagggggagc ctqctctccc ccaqqcctqt ctcctacttg aagggctctt cgggtggccc actgctctgc ccttcggggc acgttgtggg catcttccgg gctgctgtgt gcacccgggg ggttgcaaaa gcggtggact tcatacctgt tgagtctatg gaaactacca tgcggactag tagcgcttgg cgtcacccgc agttcggtgg t

#### FIGURE 1B

#### AMINO ACID SEQUENCE OF NS2/3 (810-1206)-ST (SEQ ID NO. 2)

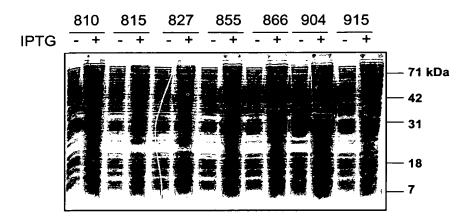
MDREMAASCG GAVFIGLALL TLSPYKVLL ARLIWWLQYL ITRVEAHLQV WIPPLNVRGG RDAILLTCA
VHPELIFDIT KLLLAIFGPL MVLQAGITKV PYFVRAQGLI RACMLVRKAA GGHYVQMAFM KLAALTGTYV
YDHLTPLQDW AHAGLRDLAV AVEPYIFSDM EVKIITWGAD TAACGDIISG LPVSARRGRE ILLGPADNFE
GQGWRLLAPI TAYSQQTRGL LGCIITSLTG RDKNQVEGEV QVVSTATQSF LATCVNGVCW TVFHGAGSKT
LAGPKGPITQ MYTNVDQDLV GWQAPPGARS MTPCTCGSSD LYLVTRHADV IPVRRGDSR GSLLSPRPVS
YLKGSSGGPL LCPSGHAVGI FRAAVCTRGV AKAVDFIPVE SMETTMRTSS AWRHPOFGG

#### A. in vitro translated N-terminal deletion mutants

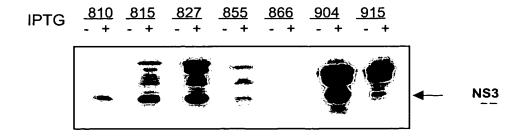
810 815 827 855 866 904 915

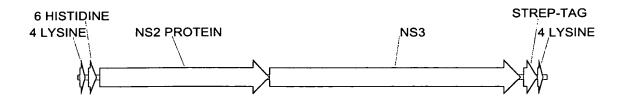
→ NS2/3 PR
→ NS3 PR

#### B. E. coli expression - SDS-PAGE

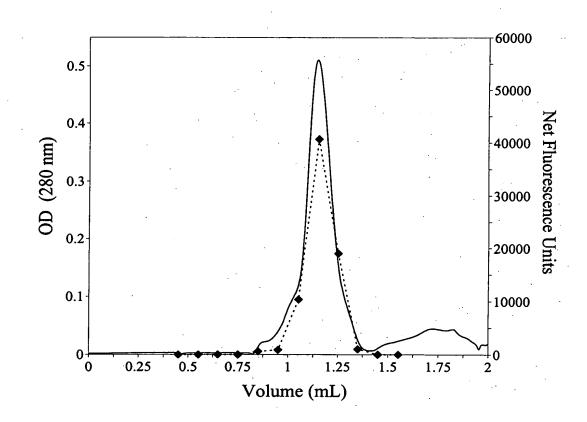


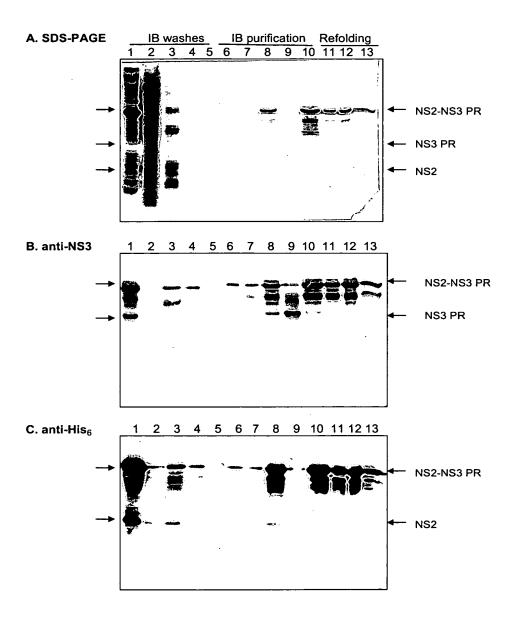
#### C. E. coli expression - Immunoblot with anti-NS3

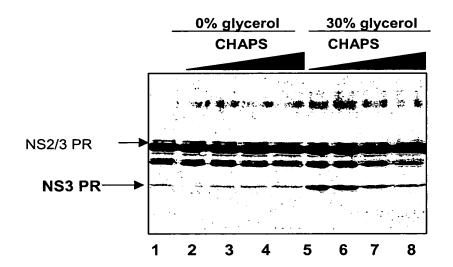


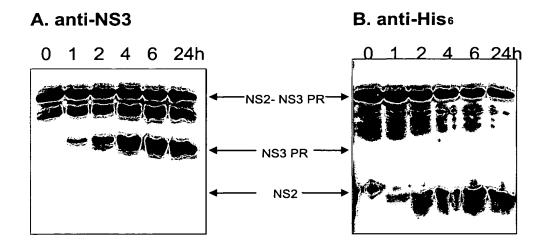


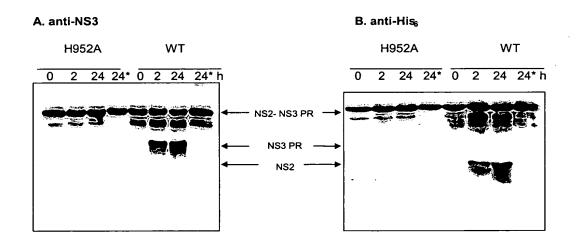
4k 6H NS2/3(904-1206)-ST-4K











#### FIGURE 9A

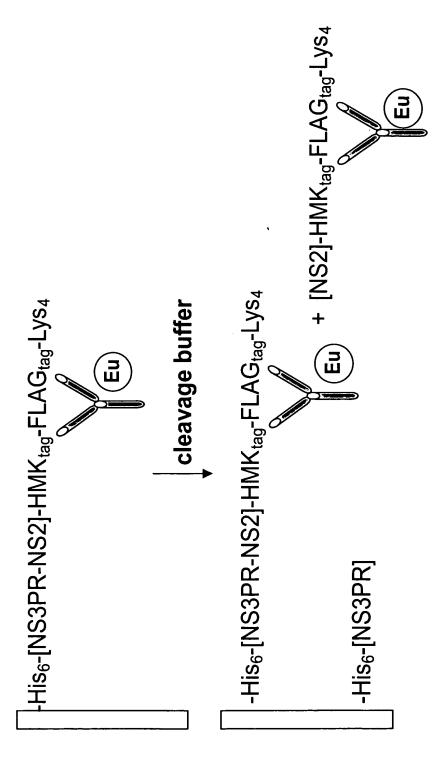
### NUCLEOTIDE SEQUENCE OF 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 3)

atgaaaaaga	aaaagctcga	gcatcaccat	caccatcaca	ctagtgcagg	cataaccaaa
gtgccgtact	tcgtgcgtgc	gcaggggctc	attcgtgcgt	gtatgttggt	gcggaaggct
gcggggggtc	attatgtcca	aatggccttc	atgaagctag	ctgcgctgac	aggtacgtac
gtttatgacc	atctcactcc	attgcaggat	tgggcccacg	cgggcctacg	agaccttgca
gtggcggtag	agcccgtcat	cttctctgac	atggaggtca	agatcatcac	ctggggggcg
gacaccgcgg	catgcgggga	catcatttca	ggtctgcccg	tctccgctcg	aaggggaagg
gagatactcc	tgggaccggc	cgataatttt	gaagggcagg	ggtggcgact	ccttgcgccc
atcacggcct	actcccaaca	gacacggggc	ctacttggtt	gcatcatcac	cagcctcaca
ggccgggaca	agaaccaggt	cgaggggag	gttcaagtgg	tctccaccgc	tacacaatct
ttcctggcga	cctgcgtcaa	cggcgtgtgt	tggactgtct	tccatggcgc	cggctcaaag
accttggccg	gccccaaagg	cccaatcacc	cagatgtaca	ctaatgtgga	ccaggacctc
gtcggctggc	aggcgccccc	tggggcgcgc	tccatgacac	catgcacctg	cggcagctcg
gacctctatt	tggtcacgag	acatgccgac	gtcattccgg	tgcgccggcg	gggcgacagt
agggggagcc	tgctctcccc	caggcctgtc	tcctacttga	agggctcttc	gggtggccca
ctgctctgcc	cttcggggca	cgttgtgggc	atcttccggg	ctgctgtgtg	cacccggggg
gttgcaaaag	cggtggactt	catacctgtt	gagtctatgg	aaactaccat	gcggactagt
agcgcttggc	gtcacccgca	gttcggtggt	aaaaagaaaa	agtaaggatc	С

### FIGURE 9B Amino acid sequence of 4K-6H-NS2/3 (904-1206) ST-4K (SEQ ID NO. 4)

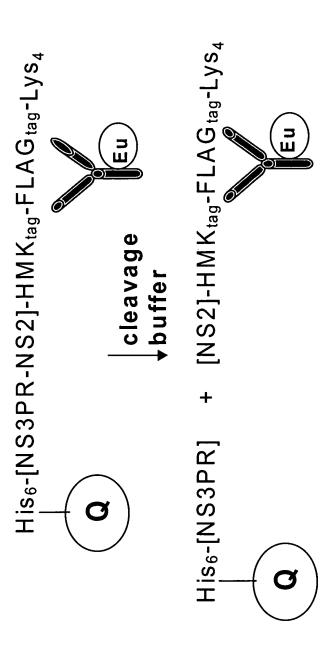
MKKKKLEHHH	${\tt HHHTSAGITK}$	VPYFVRAQGL	IRACMLVRKA	AGGHYVQMAF	MKLAALTGTY	60
VYDHLTPLQD	WAHAGLRDLA	VAVEPVIFSD	MEVKIITWGA	DTAACGDIIS	GLPVSARRGR	120
EILLGPADNF	EGQGWRLLAP	ITAYSQQTRG	LLGCIITSLT	GRDKNQVEGE	VQVVSTATQS	180
FLATCVNGVC	WTVFHGAGSK	TLAGPKGPIT	${\tt QMYTNVDQDL}$	VGWQAPPGAR	SMTPCTCGSS	240
DLYLVTRHAD	VIPVRRRGDS	RGSLLSPRPV	SYLKGSSGGP	LLCPSGHAVG	IFRAAVCTRG	300
VAKAVDFIPV	ESMETTMRTS	SAWRHPOFGG	KKKK			337





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FIGURE 11



His<sub>6</sub>-[NS3PR-NS2]-HMK<sub>tag</sub>-FLAG<sub>tag</sub>-Lys<sub>4</sub>

fluorescent probe (potent NS3 protease inhibitor)

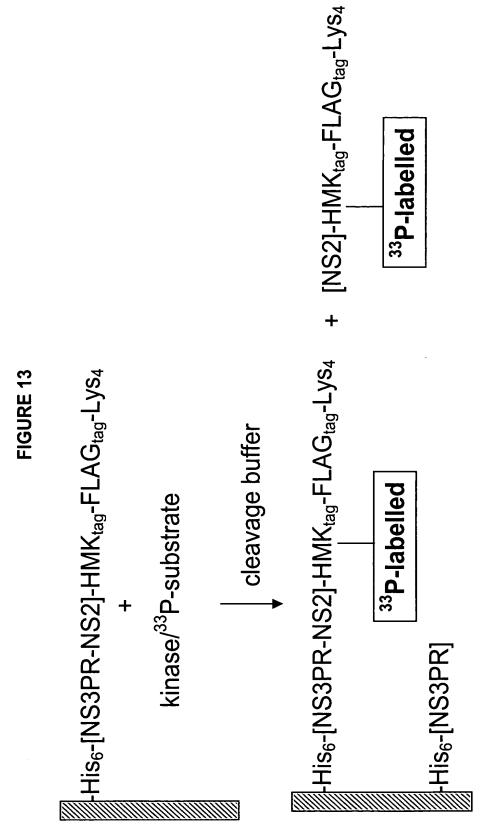
cleavage buffer

His<sub>6</sub>-[NS3PR] His<sub>6</sub>-[NS3P<sub>R</sub>-NS2]-HMK<sub>tag</sub>-FLAG<sub>tag</sub>-Lys<sub>4</sub> +

**(\*** 

+ [NS2]-HMK<sub>tag</sub>-FLAG<sub>tag</sub>-Lys<sub>4</sub>

. \* L



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Strep<sub>tag</sub>-[NS3PR-NS2]-His<sub>6</sub>-Lys<sub>4</sub>

ag\_[ινΟσί ιντινΟΖ] τι ιιος Έρνομ 50 mM Hepes, pH 7.5, 30% glycerol, 1.5 h 0.5% n-dodecyl-β-D-maltoside, 1 mM TCEP

Strep<sub>tag</sub>-[NS3PR-NS2]-His<sub>6</sub>-Lys<sub>4</sub> + Strep<sub>tag</sub>-[NS3PR] + [NS2]-His<sub>6</sub>-Lys<sub>4</sub>

transfer

Streptavidin coated plate

1 h washes

Eu<sup>+3</sup>-labeled anti-NS2 (signal decrease assay)

washes

TRF detection

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